European Review, Vol. 18, No. 3, 347–364 © 2010 Academia Europæa
doi:10.1017/S1062798710000074

Fusion Pore: An Evolutionary Invention of Nucleated Cells

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This article outlines the lecture presented by Robert Zorec at the Academia Europea meeting in Liverpool on 19 September 2008, four decades after the Sherrington Lecture of Bernard Katz who, together with his colleagues, developed a number of paradigms addressing vesicles in chemical synapses. Vesicles are subcellular organelles that evolved in eukaryotic cells 1000 to 2000 million years ago. They store signalling molecules such as chemical messengers, which are essential for the function of neurons and endocrine cells in supporting the communication between tissues and organs in the human body. Upon a stimulus, the vesicle-stored signalling molecules (neurotransmitters or hormones) are released from cells. This event involves exocytosis, a fundamental biological process, consisting of the merger of the vesicle membrane with the plasma membrane. The two fusing membranes lead to the formation of an aqueous channel – the fusion pore – through which signalling molecules exit into the extracellular space or blood stream. The work of Bernard Katz and colleagues considered that vesicle cargo discharge initially requires the delivery of vesicles to the plasma membrane, where vesicles dock and get primed for fusion with the plasma membrane, and that stimulation initiates the formation of the transient fusion pore through which cargo molecules leave the vesicle lumen in an all-or-none-fashion. However, recent studies indicate that this may not be so simple. Here we highlight the novel findings which indicate that fusion pores are subject to regulations, which affect the release competence of a single vesicle. At least in pituitary lactotrophs, which are the subject of research in our laboratories, single vesicle release of peptide signalling molecules involves modulation of fusion pore diameter and fusion pore kinetics.

https://doi.org/10.1017/S1062798710000074 Published online by Cambridge University Press

Introduction

Exocytosis, Fusion Pore and the Origin of Eukaryotic Cells

Exocytosis, the merger of a subcellular organelle membrane with the plasma membrane, is a universal process, an evolutionary invention of eukaryotic cells. The defining membrane-bound structure, which differentiates eukaryotic from prokaryotic cells, is the nucleus. However, eukaryotic cells contain other membrane-bound organelles, such as mitochondria, chloroplasts, Golgi bodies, secretory vesicles and others. How these cells evolved is a matter of speculation, since these events cannot be reproduced in the laboratory. Nonetheless, it is considered that eukaryotic cells developed from a prokaryotic-like precursor cell by endosymbiosis.[1]

While life first emerged at least 3800 million years ago, approximately 750 million years after Earth was formed, evidence for the appearance of first eukaryotic cells points to 1000 to 2000 million years ago.[1,2] The evolution of eukaryotic cells was associated with a cell volume increase by three to four orders in magnitude. The increased cell size dictated a new organizational make-up. An important reason for this is that signalling and communication within the relatively large eukaryotic cell volume could no longer be supported mainly by diffusion-based processes, which provide effective and rapid transport of molecules within the submicron range; hence, the development of subcellular organelles represented a solution for the 'signalling problem' in the relatively large volume of eukaryotic cells.

The appearance of subcellular organelles played a further, particularly important role in the development of eumetazoa (multicellular organisms). In these organisms some types of the subcellular organelles gained a special function to favour rapid communication between cells. For example, in nucleated cells chemical messengers (signalling molecules such as hormones and neurotransmitters) are stored in secretory organelles – vesicles – at high, up to almost molar, concentrations. These signalling molecules are discharged swiftly and locally from the vesicles following a triggered vesicle membrane merger with the plasma membrane that leads to the formation of an aqueous channel – the fusion pore (Figure 1). The function of secretory organelles is in the focus of biomedical research, since they take part in a process that exhibits, at least in neurons, one of the fastest known biological reactions. Moreover, not only in specialized cells, out of about 200 cell types present in the human body, the majority of these perform exocytosis in their repertoire of cellular functions. Therefore, exocytosis represents an important, yet unresolved topic in cell biology, physiology, biophysics, biochemistry, nano-engineering and many other disciplines. The understanding of this rather complex process will help solve a number of physiological and pathophysiological questions, in unicellular and in multicellular organisms from animal to plant kingdoms.

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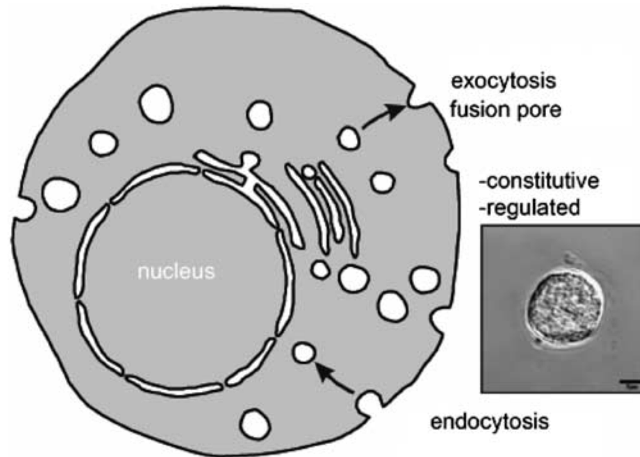

Figure 1. Exocytosis involves fusion of the vesicle membrane with the plasma membrane. This leads to the formation of the fusion pore – an aqueous channel connecting the vesicle lumen with the extracellular space. Constitutive exocytosis does not require a stimulus to occur, whereas regulated exocytosis is triggered by a stimulus, such as an increase in the activity in cytosolic Ca^{2+}. Endocytosis is a process of plasma membrane retrieval, often balancing exocytosis to keep the surface area of a cell constant over a prolonged period of time. The inset shows an isolated rat lactotroph from the anterior pituitary, prepared in culture. These cells release the hormone prolactin (scale bar = 5 μm).

Regulated exocytosis mediates a triggered release of signalling substances and fundamentally differs from constitutive exocytosis, which is believed to be a continuous, non-triggered process. Recently, there has been a substantial progress in our understanding of the molecular mechanisms underlying regulated release of vesicular signalling molecules such as hormones and neurotransmitters; however, the nature of exocytosis is still poorly understood. Among many reasons for this is the complexity of the process that involves temporally coordinated events in a spatially organized structure spanning several tens to hundreds of nanometres.

Initially, prior to actual cargo secretion, the vesicles are delivered to the plasma membrane, where they dock and get primed for fusion with the plasma membrane. Then, in response to a physiological stimulus (membrane depolarization by an action potential in excitable cells) that elevates free intracellular calcium concentration the process of exocytosis leads to the formation of a fusion pore – an aqueous channel through which secretions rapidly diffuse into the extracellular space. The central role of cytoplasmic calcium activity in triggering secretion of signalling molecules, together with questions addressing the nature of exocytosis and vesicular contents discharge, was considered by Bernard Katz and co-workers decades ago.[3]

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Calcium Hypothesis and Transient Fusion Pore Formation

Although Katz and colleagues mainly used the neuromuscular junction and the giant synapse of the squid as model systems, employing technology that prevented the study of single subcellular organelles, their results were interpreted with most interesting and still valid conclusions:

> Katz: 'R. Miledi and I now put forward a more specific suggestion: namely that depolarization opens a gate to calcium ions (or – what amounts to the same thing – that depolarization makes available specific carriers for calcium ions in the membrane). As a consequence of this increased 'calcium conductance', Ca ions can move-down a very high concentration gradient-towards the inside of the axon membrane and thus reach the critical sites of the release reaction. We are suggesting that, at these sites, calcium is essential for the process which causes a transient fusion of axon and vesicular membranes and which leads to the release of a quantal packet of transmitter.'

In experiments where the role of Ca^{2+} in triggering secretion was tested, Katz and Miledi were able to block synaptic transmission in the giant synapse of the squid by applying a pulse of strong depolarization to the presynaptic membrane.[3] Rather high depolarization made the membrane terminal at least 130 mV positive with respect to outside, thus acting as an electric potential barrier preventing Ca^{2+} entry into the cell cytoplasm. As soon as the barrier was dropped, a large surge of secretion was detected as may be observed in panels 5/6 to 8 of Figure 2 (adapted from Ref. 5, Figure 23).

This experiment provided compelling evidence to support the established current view according to which the entry of Ca^{2+} from the extracellular space is a prerequisite for the reactions that lead to the release of messenger molecules. Furthermore, the abrupt surge of secretion that followed the presynaptic depolarization also suggested that the vesicles had to be in a distinct physiological state to respond swiftly to the abrupt dissipation of the potential barrier and the ensuing increase in cytoplasmic calcium activity.

This issue is still relevant today, especially in the view of the function of the molecular machinery and the events leading to the presynaptic release. With the present knowledge of molecular structure of voltage-gated calcium channels[5] which are orders of magnitude smaller than the much more complex and much larger molecular anatomy of the secretory vesicle,[6] questions emerge of how the supramolecular structure of a vesicle can attain full and rapid release competence, and how the vesicle release can occur with such a minimal delay following the increase in cytosolic calcium activity after opening the voltage-gated calcium channels? It appears that the increased calcium activity triggers the vesicular apparatus to transit quickly from a state of no release to a state enabling the release surge of the transmitter, as described in the early experiments by B. Katz and colleagues. In particular, one needs to consider at least two scenarios of how

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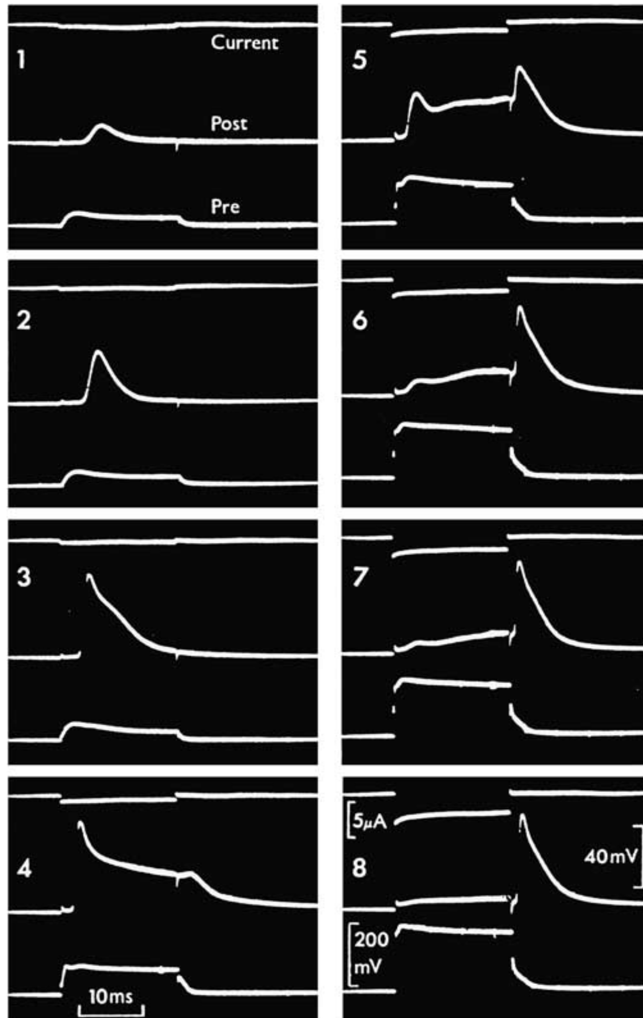

Figure 2. Suppression of transmitter release during large 'positive voltage step' of presynaptic membrane potential. Taken with permission from Katz.[3,4] Squid giant synapse treated with tetrodotoxin. Presaynaptic terminal loaded with tetraethylammonium ions. Blocks 1 to 8 display records obtained by increasing pulse intensity. In each block of records, the bottom trace shows the presynaptic voltage step, the middle record shows postsynaptic response, while the presynaptic current pulse is monitored in the top trace. Note the progressive suppression of 'on'-response (increase in postsynaptic depolarization with a short delay after the current pulse application), and replacement by 'off'-response (increase in postsynaptic depolarization at the end of the current pulse application), as presynaptic voltage is increased from 100 to about 200 mV (records 5/6 to 8).

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vesicles release their cargo swiftly in response to the stimulus: (i) a stimulus triggers a chain of reactions that include the overcoming of the high energy barrier for membrane merger and the fusion pore formation, (ii) or the vesicle and plasma membranes are already fused before the stimulus delivery, whereby vesicles are release incompetent, unless the stimulus is delivered. In the latter scenario, which intuitively exhibits a much shorter delay to a stimulus versus the first scenario, where vesicles enter the chain of events leading to membrane merger, vesicles may be pre-fused before the actual stimulus delivery, yet their pre-existing fusion pores are too narrow to mediate the release of signalling molecules.

To address these intriguing questions experimentally it would be ideal to have a model system and techniques that enable the monitoring of transitions between the functional states of a single vesicle, ranging from the release incompetent to the release competent one. One such suitable model system is that of the pituitary cells (Figure 1, inset); and the fusion pore studies undertaken in these cells will be presented in the final part of this article.

Fusion pore exhibits complex properties

Since the advent of the electrophysiological patch-clamp technique in 1982[7] it became possible to monitor time-dependent fusion of a single vesicle. This initiated a series of studies that addresses the classical view postulating that, upon stimulation, the fusion pore is formed, through which cargo molecules diffuse from the vesicle lumen into the cell exterior. Once formed, the fusion pore either closes (transient fusion), as already considered by B. Katz,[4] to allow the vesicle to be reused in the next round of exocytosis (kiss-and-run exocytosis),[8] or it fully widens leading to the complete merger of the vesicle membrane with the plasma membrane (full fusion exocytosis).[9] The patch-clamp membrane capacitance measurements[7] revealed that the fusion pore can also fluctuate between an open and a closed state in the subsecond time domain (fusion pore flickering) before full fusion of the vesicle[10] or can retain the transient nature of opening and closing for several tens of minutes or even longer, sometimes periodically ('the pulsing pore')[11] as was observed in the anterior pituitary cells[11–13] in chromaffin cells,[14] and also in plant cells.[15] In anterior pituitary cells,[11–13] rhythmic fusion pore openings and closings were sometimes recorded for relatively long periods (>600 s), which indicates that the fusion pore itself may be considered as an energetically favourable structure.

It is important to note that electrophysiological membrane capacitance measurements, where the properties of the fusion pore can be studied directly,[10,11–15] have some limitations. For example, it is difficult to reconcile that the term 'fusion pore closure' actually reflects the complete fission of a vesicle from the

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plasma membrane. Due to recording noise limitations, extreme narrowing of the fusion pore diameter may be easily misinterpreted as the fusion pore closure.[16] This consideration is important if one aims to address the physiological relevance of transient repetitive fusion events, which can be observed in some cellular systems, and in particular in the pituitary cells.

Therefore, bearing these questions in our mind, we will first discuss spontaneous and stimulated release from a single peptidergic vesicle. Then we will address the nature of the vesicle cargo release in the light of an all-or-none event as proposed by Katz and colleagues. For this we will describe measurements obtained in pituitary lactotrophs at the single vesicle level, which clearly show that vesicle discharge may not be considered simply as an all-or-nothing event. Then we will ask how partial release from a single vesicle can be attained and, finally, we will consider stimulus-dependent modulation of vesicle exocytosis.

Differences in Spontaneous and Stimulated Exocytosis

In almost all secretory cell types, including neurons[4] the basal cargo secretion, which is assumed to occur with low probability,[17] can be detected in the absence of cell stimulation. In the past, the spontaneous exocytotic events were largely neglected because of their infrequent occurrence and the belief that they exhibit similar properties as the stimulated events.[4] However, new methodological approaches revealed that spontaneous vesicle exocytosis differs from the stimulated one in many respects.

SNARE (soluble N-ethylmaleimide-sensitive factor (NSF)-attachment protein receptor) proteins were shown to mediate both spontaneous and stimulated exocytosis (reviewed in Ref. 18); however, the deletions of SNARE proteins affect spontaneous and stimulated synaptic vesicle fusion to different extents.[19–21] Some results indicate that vesicles use different SNARE components when undergoing spontaneous and stimulated fusion.[22,23] Furthermore, SNARE proteins synaptotagmin-1/-2 are thought to trigger the release of vesicle cargo by binding of Ca^{2+} upon stimulation.[24–26] On the contrary, synaptotagmin-1/-2 were shown to restrict spontaneous release.[26] Recently, synaptotagmin-12, a Ca^{2+}-independent synaptic vesicle protein controlled by cAMP-dependent processes, has been identified as a possible selective modulator of spontaneous synaptic-vesicle exocytosis.[27] Exocytotic apparatus at rest differs from the stimulated one also in the requirement of distinct proteins involved in vesicle trafficking,[28] vesicle priming,[29] and possibly vesicle recycling.[30,31] Recent studies have pointed out the role of lipids, i.e. cholesterol in balancing spontaneous and stimulated neurotransmitter release by hindering spontaneous and sustaining evoked exo-/endocytosis.[32] How sphingosine, a lipid molecule that activates the formation of the SNARE complex via synaptobrevin affects spontaneous

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exocytosis, remains to be determined.[33] Spontaneous and stimulated exocytosis differ also in the kinetics of the vesicular content discharge[12] and in the size of the fusion pore,[13] which will be discussed in the next sections.

Slow Spontaneous and Rapid Stimulated Peptide Release from Vesicles

The rate[11,34] and the amount of vesicle cargo release[35] may be regulated at the level of an individual secretory vesicle after fusion pore establishment, which has been termed post-fusion regulation.[36] To study the kinetic properties of spontaneous and stimulated peptide release,[11] we have used lactotrophs, the prolactin secreting anterior pituitary cells (Figure 1, inset).[37] It has been shown that stimulated hormone discharge from a single vesicle can be 10–20 times faster than the discharge under resting conditions (Figure 3). Optical studies revealed that a

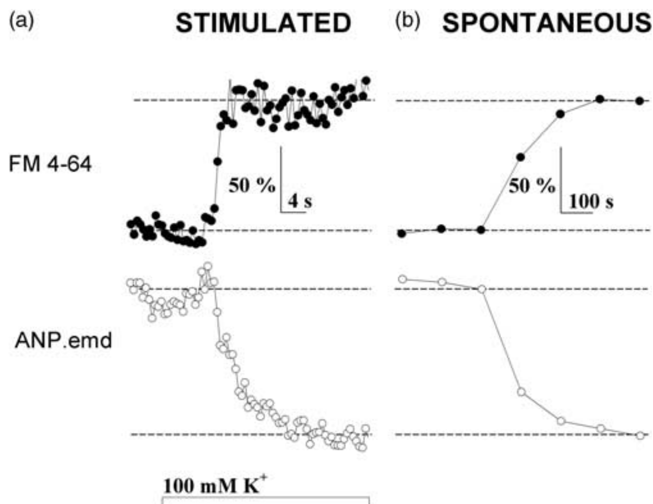

Figure 3. Rapid stimulated and slow spontaneous peptide hormone release from a single vesicle. Time-dependent fluorescent intensity changes of fluorescence peptide (ANP.emd) and styryl dye FM 4-64 indicate the release of peptides and loading of the extracellular dye into single vesicles in spontaneous and in stimulated conditions. The time-course of fluorescence intensity changes of the two fluorescent probes differ significantly; it is rather slow (minutes) in spontaneously releasing vesicles and much more rapid (seconds) in stimulated vesicles. Note that the time-course of loading and unloading is similar (synchronous) in spontaneously secreting vesicles whereas it is different in stimulated vesicles (stimulation was attained by exposing cells to a K^+-enriched extracellular solution (100 mM K^+)). Modified from Stenovec and coworkers[11] with the permission of The Federation of American Societies for Experimental Biology.

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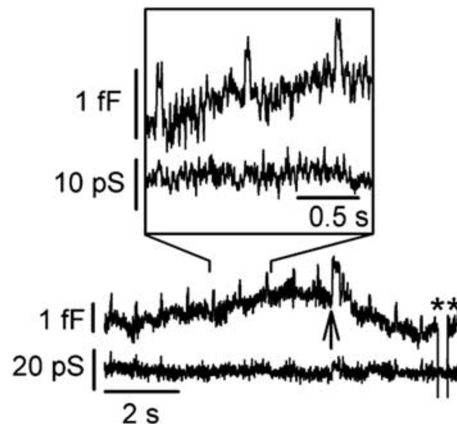

Figure 4. Repetitive transient fusion pore openings in resting lactotophs. Fusion pore openings of a single vesicle in resting pituitary cells were recorded by the patch-clamp capacitance measurements. Inset shows expanded segments of the trace, showing individual transient fusion events. Note that changes in Cm (top trace, imaginary part of admittance) in the inset are not correlated with changes in the real part of admittance (lower trace), indicating the correct phase angle setting of the lock-in amplifier by the calibration pulse (**) at the end of the trace. Arrow indicates a fusion pore event with higher amplitude and a lower frequency of occurrence (appearing every ~12 s), suggesting the presence of two vesicles in this cell-attached membrane. Note the pulsing behaviour of the fusion pore with fusion pore dwell-time of around 50 ms. Adapted from Stenovec and coworkers[11] with the permission of The Federation of American Societies for Experimental Biology.

fluorescently tagged peptide (ANP.Emd), similar in size to the hormone prolactin, is released from prolactin vesicles within seconds after stimulation. Simultaneously, vesicle loading with the styryl dye FM 4-64 was observed. On the contrary, the hormone release and the FM 4-64 loading was slow (~3 min) in many spontaneously releasing vesicles, indicating that the fusion pore properties in resting and in stimulated conditions differ (Figure 3).[11]

Measurements of cell-attached membrane capacitance in resting lactotrophs revealed regular repetitive transient fusion pore openings (the phenomenon termed 'the pulsing pore'), appearing in bursts with the duration up to 760 s.[11–13] The duration of a single transient event at rest was ~50 ms (Figure 4)[11,12] similar to the resting synapses of calyx of Held.[38] It has been proposed that the slow exchange of fluorescence probes through the fusion pore in resting lactotrophs (Figure 3) may be constrained kinetically by a long-lasting regular fusion pore gating.[11] Transient vesicle fusion can limit or prevent peptide hormone release because of the large molecular size of peptides and the consequent low diffusional mobility of peptide molecules.[39–41] Similarly, fusion pore flickering can limit

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dopamine release from synaptic terminals in neurons.[42] This indicates that despite the relatively high diffusional mobility of small chemical transmitters, fusion pore gating may also be involved in the regulation of small chemical transmitter release in synapses.[43]

Interestingly, the long lasting repetitive transient fusion pore openings recorded in physiological experiments indicate that a vesicle is stably fused to the plasma membrane. Therefore, one may be able to see such a structure by electron microscopy even in resting conditions. Indeed, scanning electron microscopy studies in which the surface structure of the individual resting pituitary cells was monitored revealed tiny openings, which may represent structures related to the fusion pores observed in electrophysiological measurements (Figure 5).

Subnanometre Fusion Pore Diameters at Rest Expand upon Stimulation

The slower vesicle cargo release observed in resting lactotrophs (Figure 3) might also reflect a narrow fusion pore of spontaneously releasing vesicles. Vesicle fusion was monitored by confocal microscopy in resting and stimulated lactotrophs expressing synaptopHluorin (spH), a pH-sensitive green fluorescence protein that enables optical discrimination between unfused and fused vesicles.[44] Simultaneously, the permeation of FM 4-64 dye and HEPES molecules through the fusion pores was monitored.[12] Fusion pore openings associated with a change in spH fluorescence, were observed in >50% of the spontaneous exocytotic events.[12] In these events the fusion pore was permeable to protons, as indicated by spH fluorescence changes, but impermeable to FM 4-64 (left; molecular diameter = ~0.9 nm) and HEPES (molecular diameter = ~0.5 nm) molecules. Thus, the fusion pore diameter in many resting peptidergic vesicles is <0.5 nm, which is smaller than the size of peptide hormone prolactin stored in these vesicles (prolactin molecular diameter = ~5.2 nm).[12] Membrane capacitance measurements confirmed subnanometre fusion pores in resting lactotrophs[12,45], suggesting that exocytosis without release of vesicle cargo may occur before delivery of the stimulus ('unproductive exocytosis', meaning that vesicles are release incompetent).

Upon stimulation, >70% of exocytotic events exhibited a larger, FM 4-64–permeable pore (>0.9 nm) consistent with previous fusion pore permeation studies.[39,40,46,47] In capacitance measurements, stimulation decreased the fraction of events with lowest measurable fusion pore diameter of 3.2 nm from 25% to 2% reflecting much wider pore diameters upon stimulation and likely a massive vesicle cargo discharge in the majority of these events.[12] Similarly, fusion pore expansion was observed in chromaffin cells upon stimulation, which likely improves the efficiency of release of small classical transmitters.[47,48] Moreover,

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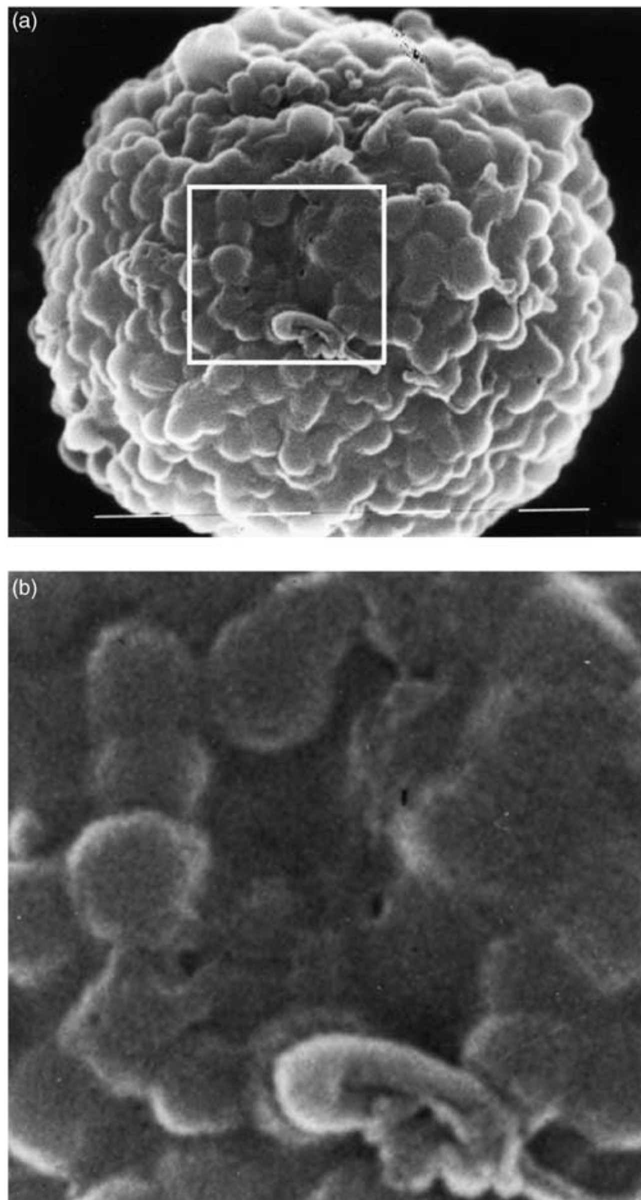

Figure 5. Scanning electron micrograph of the pituitary lactotroph surface exhibits fusion-pore-like formations. Panel A shows a view of the whole cell with a diameter of 10 μm. Panel B is magnified view of the framed region in panel A. Several small openings in the cell surface (black), which may be related to the fusion pore-like structures, are noticeable.

in stimulated lactotrophs, the pore size can reversibly open to several nanometres in diameter, since quantum dots coupled to prolactin antibodies can be introduced into retrieving prolactin vesicles.[49]

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Stimulus Modulates Fusion Pore Kinetics

While transient fusion is accepted as a mode of vesicle cargo release, both in central neurons and neuroendocrine cells, the prevalence of this mechanism in comparison to full fusion is still under discussion.[50] In lactotrophs, transient vesicle exocytosis appears to be the predominant mode of exocytosis in spontaneous and in stimulated cells.[12] Optical studies on lactotrophs expressing spH revealed that 65% of spontaneous events were transient, some of them were repetitive.[12] In 35% of all events, spH fluorescence persisted after fusion for >100 s. On the contrary, the majority of stimulated events ($>93\%$) were persistent.[12] In persistent events the lateral diffusion of spH signal from the site of exocytosis into the plasma membrane was not detected,[12] suggesting that vesicle exocytosis occurred in a non-full-collapse mechanism.[51,52] Since FM 4-64 loading was fourfold slower in persistent events than in transient events, spH signal likely persisted because of a rapidly flickering fusion pore[11,42] and not because of a long open fusion pore lifetime.[53] This hypothesis was confirmed by capacitance measurements, which revealed that transient fusion pore openings with the burst duration of >100 s are the predominant mode of spontaneous and stimulated exocytosis in lactotrophs.[11,12] Optical monitoring of fusion pore dynamics is limited by the characteristics of the spH construct. Thus, the effect of fusion pore closure is poorly reflected in these studies because of vesicle reacidification, which in comparison to the fusion pore closure observed as an off-step in capacitance measurements is rather slow ($\tau = 4$–5 s for synaptic vesicles).[54] These results are in contrast to previous studies on chromaffin cells where increased cell stimulation shifted the mode of exocytosis from transient to full fusion, resulting in the modulation of the amount of cargo release from a single vesicle.[47,55,56]

Capacitance measurements also revealed that, in lactotrophs, transient vesicle fusion occurred four times more frequently after stimulation with a twofold longer fusion pore dwell-time and a wider pore diameter.[12] Therefore, a stimulus prolongs the effective opening of a fusion pore and expands its subnanometre diameter to enable hormone secretion without full fusion.[12]

Perspectives

In contrast to the views developed by Bernard Katz decades ago, where transient fusion was considered to be an event mediating complete discharge of the vesicle content, recent findings indicate that vesicle content may be emptied incompletely. A key role in partial vesicle discharge appears to occur at the post-fusion stage. Fusion pores are subject to physiological regulation and this affects the amount of vesicle cargo to be released from a single vesicle. Therefore, vesicles may exhibit different release competence. Current knowledge of mechanisms determining vesicle release competence include fusion pore diameter regulation

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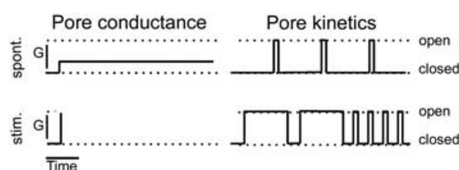

Figure 6. Fusion pore properties modulation before and after stimulation. The permeation of molecules through the fusion pore depends on fusion pore conductance (G; diameter) and/or fusion pore kinetics. A wider fusion pore (higher G) and/or faster frequency of fusion pore openings with longer fusion pore dwell-times (faster kinetic of fusion pore openings) leads to a faster, more effective release of peptides from vesicles in stimulated events. Modified from Stenovec and coworkers[11] with the permission of The Federation of American Societies for Experimental Biology.

as well as the fusion pore dwell-time determination.[11,36] In lactotrophs, the experiments point to the existence of both mechanisms: release of vesicle cargo may be restrained kinetically and/or due to a narrow fusion pore. Under stimulation, a preformed fusion pore may retain the transient nature, but with a prolonged dwell-time, increased frequency of re-openings and an increased fusion pore diameter. All of these changes will facilitate the vesicle cargo release (Figure 6).

A key question to be answered in the future is related to the nature of the repetitive transient fusion events. Do they represent cycles of merger/fission of a single vesicle? Given that membrane merger involves high energy barrier, such a repetitive cycle is unlikely. Perhaps the transient fusion events represent fluctuations of an open fusion pore between the states where the pore is extremely narrow, with a neck formed by the fused vesicle and plasma membrane. A narrow fusion pore may structurally involve membrane domains with high local curvature, which may be attained by highly anisotropic molecules with negative curvatures.[57] In support of such a distinct membrane structure adjacent to fused vesicles versus plasma membrane, it was reported that plasma membrane sites where prolactin vesicles engage into exocytosis are distinct from the plasma membrane areas devoid of docked prolactin vesicles.[58] The basis of energetic stability of such special curved structures is to be addressed in the future as well. Finally, the role of SNARE proteins in these physiological states remains to be determined as well.

Acknowledgements

This work was supported by grants P3 310 381, Z3 7476 from the Ministry of Higher Education, Sciences and Technology of the Republic of Slovenia, NS36665 from the National Institutes of Health, EC grant No. QLG3 2001-2004, EC support DECG, CLG3-CT-2001-02004, and EC GROWBETA QLG1-CT-2001-02233.

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About the Author

Robert Zorec is head of the national research programme 'Cell physiology', funded by the Research Agency of Slovenia, and has been Professor of Pathophysiology at the University of Ljubljana since 1995. He received training in classical biology, animal physiology and electrophysiology at the University of Ljubljana and the Medical School of the University of Newcastle upon Tyne, where he started to use the 'patch-clamp technique' to monitor currents through single ion channels. After a Wellcome Trust position at the University of Cambridge and Babraham, UK, he introduced the membrane capacitance measurements to study a number of secretory cells also at the level of the elementary fusion event. He is a fellow of the Slovenian Academy of Sciences and Arts and of the Academia Europea. One of the main topics of research of the Laboratory of Neuroendocrinology-Molecular Cell Physiology and of the Laboratory of Cell Engineering is to understand the molecular nature of regulated exocytosis, a process ubiquitous to eukaryotic cells, consisting of the vesicle membrane fusion with that of the plasma membrane, but still poorly understood. In the present article key properties of this important biological process are reviewed by the research colleagues of R. Zorec.

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